Human sICAM-1 ELISA Kit

Cat #: orb1085878 (manual)

Size: 96 tests

For research use only, not for clinical diagnosis.

This kit is used to quantify the amount of sICAM-1 in samples such as human serum, plasma, or cell culture supernatants. Please read the manual carefully and check the reagent components before use. If you have any questions, please contact biorbyt, we will provide you with technical support.

Assay Principle

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Purified sICAM-1 antibody was coated in the microplates, then the standard or the samples to be tested are added to the coated plate wells. After the antigen fully reacts with the coated antibody, the biotinylated sICAM-1 antibody and streptavidin labelled with HRP are added in turn. The biotin and streptavidin form high-intensity non-covalent binding. After sufficient washing, the substrate TMB is added for colour development. TMB is converted to blue under the catalysis of HRP enzyme and finally to yellow under the action of stop solution. The colour shading is proportional to the sICAM-1 content in the sample. Finally, the OD value was determined by microplate reader at the wavelength of 450 nm, and the concentration of sICAM-1 in the sample was calculated by plotting the standard curve.

Kit Components

Reagents	Specifications（96T）	Storage Conditions
Antibody-Coated Slats	8×12	2-8°C
Standard	2 tubes	2-8°C
S1 Standard/Sample Dilution Buffer	16 ml×4 bottle	2-8°C
Biotin-Labelled Antibody (Concentrated, 100×）	60µl×2 bottles	2-8°C
S2 Biotin-Labelled Antibody Dilution Buffer	16ml×1 bottle	2-8°C
HRP-Streptavidin Conjugate (Concentrated, 100×）	60µl×2 bottles	2-8°C
S3 HRP-Streptavidin Conjugate Dilution Buffer	16ml×1 bottle	2-8°C
Washing Buffer （Concentrated, 20×）	25ml×1 bottle	2-8°C
TMB Substrate （Avoid direct light）	12ml×1 bottle	2-8°C
Stop Solution	12ml×1 bottle	2-8°C

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Plate Sealer	4 pieces	
Manual	1 copy	

Required Instruments and Reagents

1. Microplate reader (Main wavelength: 450 nm, reference wavelength: 630 nm.)
2. Precision single (0.5-10μL, 2-20μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips (calibration is required before use.)
3. Automated plate washer
4. 37°C incubator
5. Deionized or distilled water
6. Coordinate paper
7. Measuring cylinder

Precautions

1. The kit is stored in 2-8℃ and the dissolved but unused standard is recommended for disposal. Do not mix kit components from different sources or batch numbers, use this product within the expiration date.
2. When the concentrated washing solution is taken out at low temperature, there may be crystal precipitation, and it can be dissolved by heating in the water bath before dilution, which does not affect the use.
3. Pipettes should be calibrated before and used for each step and to avoid errors. It is recommended to control the sampling time within 5 minutes. If there are a large number of samples, it is recommended to use a multi-channel pipette for sampling.
4. Run a standard curve with each measurement, ideally in duplicate. If the target analyte concentration in a sample exceeds the upper detection limit of the kit (sample OD > first standard OD), dilute the sample with sample diluent by an appropriate factor (n-fold) prior to measurement and multiply the result by the total dilution factor.
5. To avoid cross contamination, remember to replace the tip when adding different concentrations of standard, different samples and different reagents. The plate sealer is disposable.
6. The concentrated HRP conjugate and TMB substrate should be protected from light. The TMB substrate should be kept colorless before adding. Do not use the TMB substrate when it turned blue.
7. Strictly follow the manual, and the test results must be based on the microplate reader reading.

Sample Collection and Storage

1. **Serum**: Blood coagulated naturally at room temperature for 30 min and centrifuged for 20 min (2000-3000 rpm). Collect supernatant carefully. If precipitation occurs during storage, centrifuge again to avoid repeated freezing and thawing.
2. **Plasma**: Select EDTA or citric acid as anticoagulant according to sample requirements and centrifuge for about 20 min (2000-3000 rpm). Collect the supernatant carefully and centrifuge again if precipitation occurs during storage.

3. **Cell supernatant**: When detecting secretive components, collect with a sterile tube. Centrifuge for about 20 min (2000-3000 rpm). Collect the supernatant carefully.

4. If the sample cannot be tested immediately, dispense it according to the minimum amount of use, and store it in -20℃-70℃ to avoid repeated freezing and thawing. Avoid haemolytic or hyperlipidaemia samples. If the serum contains a large amount of particles, centrifuge or filter to remove them before testing; Thaw at room temperature, do not heat thaw at 37℃ or higher.

5. Samples containing NaN3 could **not** be tested because NaN3 inhibited horseradish peroxidase activity.

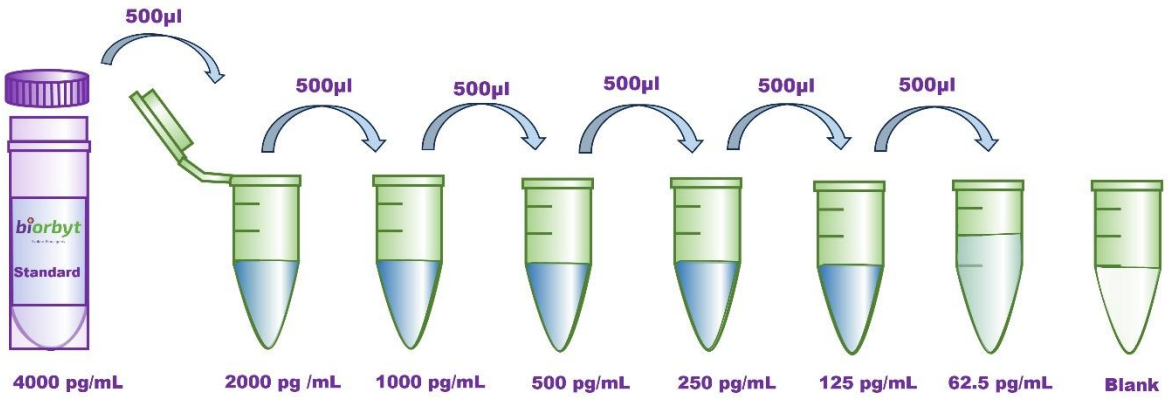
6. Please dilute the sample in appropriate fold according to the actual situation (it is recommended to determine the dilution fold according to the pre-test results).

Reagent Preparation

1. Reagent equilibration: Please reheat the reagent kit and the sample to be tested at room temperature within 30 minutes before the test.

2. Preparation of Washing Buffer: Dilute the concentrated Washing Buffer (20×) to Washing Buffer working solution (1×) with double distilled water or deionized water, and keep it as standby.

3. Gradient dilution of standard: Take 1 ml of Standard/Sample Dilution Buffer (S1) into the lyophilized standard, allow it to stand for 15 min until it is completely dissolved, then gently mix it with a concentration of 4000pg/ml, take 6 EP tubes, add 500ul Standard/Sample Dilution Buffer (S1) each EP tube, and dilute twice according to the following concentration: 2000, 1000, 500, 250, 125, 62.5pg/ml were diluted. 4000pg/ml is the highest concentration of the standard curve, and the Standard/Sample Dilution Buffer (S1) is the zero point (0pg/ml) of the standard curve. The Standard Stock Solutions (4000pg/ml) that has not been used up should be discarded or dispensed as required in one dose and stored in the -20~-80℃ refrigerator.



4. Working solution of Biotin-Labelled Antibody: Dilute the concentrated Biotin-Labelled Antibody (100×) to working solution (1×) with Biotin-Labelled Antibody Dilution Buffer (S2) according to the required amount of the test, and use it within 30 min.

5. Working solution of HRP-Streptavidin: Dilute the concentrated HRP-Streptavidin (100×) to working solution (1×) with HRP-Streptavidin Conjugate Dilution Buffer (S3), and use it within 30 minutes.

Operation Steps

1. Sample adding: Take out the Antibody-Coated Slats according to the required amount of the test, set one well as the blank control well, and respectively add 100µl of the prepared standards, standard zero point (S1) and the samples to be tested to the bottom of the wells.

2. Incubation: Seal the plate with the Plate Sealer, incubate for 90 min at 37℃ (except for blank control wells).

3. Washing: Carefully remove the Plate Sealer, discard the liquid, spin dry, fill each well with 1× Washing Buffer (350 µl), allow to stand for 30s and then discard. Repeat 4 times, and finally dry on absorbent paper.

4. Biotin-Labelled Antibody adding: Add 100µl Biotin-Labelled Antibody working solution to each well, except for blank control wells.

5. Incubation: Seal the plate with the Plate Sealer, incubate for 60 min at 37℃, except for blank control wells.

6. Washing: Same as the above washing process (Step 3), wash the plate for 4 times.

7. HRP-Streptavidin adding: Add 100µl HRP-Streptavidin working solution to each well, except for blank control wells.

8. Incubation: Seal the plate with the Plate Sealer, incubate for 30 min at 37℃, except for blank control wells.

9. Washing: Same as the above washing process (Step 3), wash the plate for 4 times.

10. Colour development: Add 100µl of TMB substrate to each well, and Seal the plate with the Plate Sealer and then start colour development for 10-20 min at 37℃.

11. Termination: Add 100µl of Stop Solution to each well (at this time, blue turns to yellow).

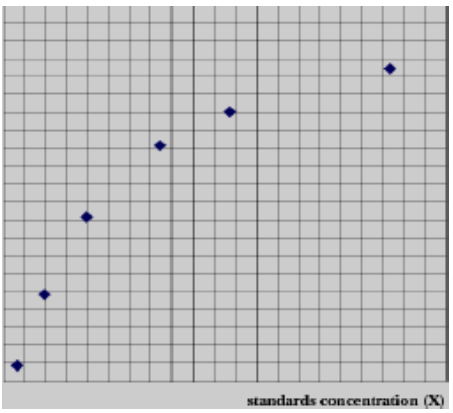
12. Determination: Measure the absorbance (OD value) of each well at the microplate reader with a wavelength of 450nm. The measurement should be performed within 5 min after adding the stop solution.

Result Judgment

1. The OD value of each standard and sample minus the OD value of the blank well is the final value, and if a duplicate well is made, its mean value shall be calculated.

2. Take the absorbance OD value as the ordinate (Y) and the corresponding standard concentration as the abscissa (X) to generate the corresponding standard curve. The sICAM-1 content of the sample can be calculated from the standard curve according to its OD value. If the OD value of the sample is higher than the upper limit of the standard curve, perform the appropriate dilution during the test, and then multiply the concentration of the sample by the corresponding dilution.

This picture is for reference only and shall be based on the standard curve drawn in the actual test.



Kit Performance

Intra-assay and inter-assay coefficients of variation are less than 10%.

Detection Range

62.5 pg/ml -4000 pg/ml

Sensitivity

31 pg/ml

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Troubleshooting

Problems	Possible Causes	Solutions
No signal	Mixing Reagents with Different ELISA kits or Batch Numbers	Recheck the label of the reagents to make sure that all components are in the testing kit being used. Do not mix reagents of different testing kits or batch numbers.
	Missing antibody, enzyme and chromogenic agent	Check the operation procedure, and be careful not to omit adding.
	HRP enzyme contaminated with sodium azide	Re-preparation of reagent, no sodium azide
	Wrong reagent preparation/use	Redo the test, operate in strict accordance with the manual, and see the labels clearly before each preparation and use
Weak signal	Reagents Expires expiration date	Check product validity
	Insufficient incubation time	Check the incubation time
	Use of contaminated reagents	Check if reagent is contaminated
	Incorrect instrument setting, filter mismatch	Whether the instrument is set correctly and the filter is used, etc.
	Washing operation is not standard	If the washing is insufficient, increase the number of washing times or extend the washing time
		Wash the bottle, each well shall be completely filled with washing buffer, and pour out quickly
		If a plate washer is used, it shall be calibrated and set to a volume sufficient to fill each hole and the inside of the plate shall not touch the equipment
		Check whether there is residual washing liquid in each well or the volume of sample added in each well is accurate
		You can add a 30 second soak between washings
High background	Improper incubation temperature and time in the experiment	Determine the appropriate incubation temperature and time for each test step
	Excessive enzyme addition	Check whether the regulating amount of pipette is correct before adding enzyme
		Check dilution and perform titer determination if necessary
The standard curve is good, but the sample wells have no signal	Low content of target in sample or no target in sample	Set the positive control and repeat the experiment
	Sample matrix effect influence detection	Test again after re-diluting sample
The standard curve is good, but the sample wells have high signal	The content of sample to be tested exceeds the standard curve range	Test again after re-diluting sample

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